

REMARKS

This Reply is in response to the Office Action dated October 20, 2004. Reconsideration and reexamination of the subject application, as amended, pursuant to and consistent with C.F.R. § 1.112, and in light of the remarks which follow respectfully requested.

By the present amendments, the claims have been amended in order to expedite prosecution. Particularly, all the claims specify that the assay or assay kit screens for compounds that modulate G_i protein induced signaling pathways. As explained in the subject application, this invention involves the unexpected discovery that T1Rs and T2Rs are able to functionally couple via G_i proteins in cells which endogenously express or which may be genetically engineered to express such G_i proteins. For the reasons set forth *infra*, the references fail to teach or suggest such assays and assay kits. It is anticipated that the present amendments shall place the case in condition for allowance.

Turning to the Office Action, the oath stands objected to as being improper. This objection is overcome based on the Application Data Sheet submitted herewith which indicate the mailing addresses of the inventors. The citizenship of Guy Servant and Hong Xu have been revised to correspond to the declaration previously submitted. Withdrawal of this objection is respectfully requested.

Claims 3 and 57 stand objected to based on the recitation "Hela" instead of "HeLa". This objection is moot in view of the present amendments.

Claim 1 stands rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3, 8 and 10 of US Patent No. 6,558,910. This rejection is respectfully traversed to the extent it may be applicable to the claims as amended herein.

Applicants respectfully advise that the claimed assays screen for modulators of taste receptor GPCR's, particularly T1R or T2R taste receptors by assaying the effect of a candidate compound on a G_i protein signaling pathway. As discussed in the present application, it has been unexpectedly discovered that T1Rs and T2Rs couple to G_i proteins and have developed novel assays based on this discovery. Particularly, it has been shown that the coupling of T1Rs or T2Rs via G_i proteins results in the inhibition of the adenylyl cyclase enzyme and a decrease in cAMP levels within cells wherein such G_i mediated coupling occurs.

As disclosed in the subject application, this coupling was totally unanticipated and was not obvious based on what had then been known about T1R and T2Rs. Particularly, whereas it had been previously reported that gustducin as well as promiscuous G proteins or variants thereof such as $G_{\alpha 15}$ and $G_{\alpha 16}$ functionally couple to T1Rs and T2Rs, these G proteins are not G_i proteins and do not trigger G_i -mediated signaling pathways.

More specifically, the coupling of T1Rs and T2Rs via promiscuous G proteins or variants result in the activation of an enzyme, PLC, and calcium mobilization within cells. By contrast, the coupling of T1Rs or T2Rs via G_i proteins, as noted *supra* results in the inhibition of a different enzyme, adenylyl cyclase and a decrease in cAMP levels mediate within cells. This G_i cAMP response (reduction) is not calcium dependent as it relies on a

totally different signaling cascade from that used by promiscuous G proteins. Indeed, the G_i signaling pathways on the effects on cAMP measured in the claimed methods do not involve calcium mobilization. Moreover, taste receptors do not promote calcium mobilization in the absence of promiscuous G proteins or their variants. (See e.g., Chandrashekar et al., *Cell* 100:703-711 (2000) and Nelson et al., *Cell* 106:381-390 (2001)).

Based on at least these differences the '910 Zucker patent does not teach or suggest the claimed assays. Rather, while the patent prophetically discloses assays to identify modulators of the claimed taste receptor, there is absolutely no mention that these receptors functionally couple via G_i proteins. Rather, the only G proteins prophetically mentioned in the '910 patent are promiscuous G proteins such as $G_{\alpha 15}$ and gustducin. However, as noted above, these G proteins couple and trigger completely different signaling pathways than G_i proteins.

Moreover, it was not anticipated or obvious based on the fact that promiscuous G proteins couple to taste receptor GPCRs that G_i proteins would as well. For example, it has been found that two other types of G protein, G_s and G_q proteins do not functionally couple T1Rs and T2Rs. Indeed, the very reason that scientists have had to resort to the use of promiscuous G proteins in assays that screen for modulators of T1Rs and T2Rs is the fact that these receptors do not couple via G_s or G_q proteins. (See, e.g., Figure 7 in Chandrashekar et al. *Cell* 100:703-711 (2000)). Consequently, in prior assays researchers have had to co-transfect sequences which encode for a particular taste GPCR as well as a promiscuous G protein to monitor taste receptor activities. (See Chandrashekar et al., *Cell* 100:703-711 (2000); Nelson et al. *Cell* 106:381-390 (2001) and Li et al.).

Therefore, because the '910 patent is silent with respect to the coupling of taste receptors via G_i proteins, and further because it was unpredictable based on what had been reported in the art with respect to other types of G proteins, withdrawal of the double patenting rejection based on the '910 patent is respectfully requested.

Claims 1-4 and 15-21 stand provisionally rejected as being obvious over claims 116-120, 122, 123, 127, 128, 130-132 and 142 of copending application US Serial No. 10/725, 472 ("the Zoller application" or "the '472 application").

For similar reasoning, Applicants respectfully submit that the Zoller application claims do not teach or suggest the claimed assays. As noted above, the present claims identify T1R or T2R modulators based on their effect on G_i protein-mediated signaling pathways. By contrast, the Zoller patent application (and supporting examples) identify T1R or T2R modulators in assays which screen for compounds that modulate promiscuous G protein (*e.g.*, $G_{\alpha 15}$ or $G_{\alpha 16}$) mediated signaling pathways.

As noted above, promiscuous G proteins do not activate the same signaling pathways as G_i proteins. In fact, they operate on totally different enzymes. Whereas coupling of taste receptors GPCRs via promiscuous G proteins or variants results in a activation of the PLC enzyme (resulting in calcium mobilization (influx) within cells), taste receptor coupling via G_i proteins instead results in an inhibition of a different enzyme, adenylyl cyclase, and a decrease in cAMP. This latter response is not calcium dependent and does not involve calcium mobilization.

Therefore, the claims of the cited Zoller application do not anticipate or render obvious the claimed assays. Withdrawal of the § 101 double patenting rejection of claims 1-4 and 15-21 based on the '472 application is therefore respectfully requested.

Claims 8, 24-25, 46, 58 63 and 65 further stand rejected under 35 U.S.C. § 112 second paragraph. This rejection is respectfully traversed to the extent it may be applicable to the claims as amended.

The antecedent basis rejection of claim 8 is moot as the improper antecedent basis for "said monoclonal antibody" has been corrected.

The objection to claims 24 and 25 with respect to tradenames is moot as the tradenames are no longer recited in the claims.

The objection to claim 46 has been cured as there is now proper antecedent basis for the recited immunoassay.

The objection to claim 58 is cured as well because the claim has been amended to provide proper antecedent basis for the assay.

Claims 1-4, 9-13, 15-20, 23, and 47-49 stand rejected under 35 U.S.C. § 102(e) as assertedly being anticipated by the cited Zoller patent application.

This rejection is respectfully traversed to the extent it may be applicable to the claims as amended.

As noted above, the subject assays identify modulators of T1Rs or T2Rs, by screening the effect of candidate compounds on G_i proteins mediated signaling pathways. By contrast,

the cited Zoller patent application instead teaches and exemplifies assays that screen for T1R or T2R modulatory compounds, based on their effect on promiscuous G protein mediated signaling pathways. This is clear from the text of the Zoller patent application relied upon by the Examiner in the Office Action. For example, claim 20 recites that the cells are transfected with an appropriate G protein (promiscuous G proteins) which results in the activation of certain signaling pathways.

By contrast, the present invention claims assays which monitor completely different signaling pathways, and consequently will result in the identification of different modulatory compounds.

As noted above, it was not anticipated or obvious from the Zoller application that G_i proteins would have functionally coupled T1R or T2R taste receptors, especially in view of the fact that other types of G proteins (G_s and G_q) do not functionally couple these taste receptors. Essentially, the fact that cells endogenously express a particular type of G protein, *e.g.*, a G_i protein, is insufficient to suggest that this G protein will be capable of functionally coupling to a particular GPCR, absent supportive experimental data or a scientific basis to reasonably anticipate this result. Indeed, cells may express multiple GPCRs which may or may not couple via G_i mediated signaling pathways. Herein, the Zoller patent application does not provide such experimental results. Moreover, the existing state of the art would have suggested that G_i coupling would not occur since G_s and G_q proteins do not couple T2Rs or T2Rs.

Therefore, based on the foregoing, withdrawal of the § 102(e) rejection of claims 1-4 and 9-13, 15-20, 23 and 47-49 based on Zoller et al respectfully requested.

Claims 26-29, 37-42, 61-63 and 65 further stand rejected under U.S.C. § 103(c) as being unpatentable over same Zoller et al. patent application, particularly the disclosure contained in claims 161-178. These claims are directed to assays that screen for T1R taste modulators, with cAMP assays being mentioned in paragraph [0070] of the Zoller patent application.

This obviousness rejection is respectfully traversed. Again, Applicants respectfully submit that the Zoller patent application, while admittedly disclosing that T1Rs functionally couple via G proteins including promiscuous G proteins, and activate signaling pathways induced thereby, does not teach or suggest that these receptors would functionally couple via G_i proteins. That T1Rs and T2Rs functionally couple via promiscuous G proteins would not suggest the claimed methods, because, as noted above, promiscuous G proteins act on totally different signaling pathways (and act on different enzymes) relative to G_i proteins. Also, the state of the art at the time of filing of this application supports a conclusion that it was non-obvious that G_i proteins would be capable of coupling to T1Rs and T2Rs, especially given the fact that several other types of G proteins, in G_q and G_s proteins, do not. This establishes the inherent unpredictability concerning whether a particular G protein will functionally couple to a particular GPCR.

Therefore, based on the fact that Applicants are claiming functionally different assays than disclosed or claimed in the Zoller '472 application, which are non-obvious based thereon, withdrawal of the obviousness rejection of claims 26-29, 37-42, 61-63 and 65 is respectfully believed to be in order.

Claims 33-35 stand rejected under U.S.C. § 103(a) as assertedly being obvious over Zoller et al. patent application in view of Li et al.

The Zoller application is applied as previously. Li et al. is cited based on its disclosure of assay methods wherein HEK-293T cells are transfected with T1R2/T1R3 and $G_{\alpha 14}$ chimeras. The Office Action notes that these chimeric G proteins yield intracellular calcium increases, and therefore functionally couple to the T1R2/T1R3 taste receptor.

This rejection is also respectfully traversed. As appreciated by the Examiner, the application is silent with respect to the fact that T1Rs functionally couple with G_i proteins and activate G_i protein induced signaling pathways. The deficiency of the Zoller application is not cured by the Li et al reference.

By contrast, Li et al. reports the use of variants of a promiscuous G protein ($G_{\alpha 15}$) which is modified at its terminus to include 5 amino acids of other G proteins, *e.g.*, $G_{\alpha i3}$. However, these promiscuous G protein variants do not activate G_i mediated pathways. Rather, the coupling of these promiscuous G proteins or variants results in the activation of the PLC enzyme, which in turn results in an increase in intracellular calcium. This is clear from the Li et al. (*Id.*) reference. As noted *supra*, the subject assays are calcium independent.

By contrast, it is clear from the Li et al. reference that the observed results require co-transfection of T1R2/T1R3 and the exogenous variant promiscuous protein, and that this results in an influx of intracellular calcium. Therefore, Li et al. monitor a completely different signaling pathway than the claimed invention. By contrast, the inventive assays monitor the effects of putative T1R or T2R modulations on G_i mediated T1R or T2R receptor activation, wherein such G_i protein may be endogenously expressed by the test cell, *e.g.*, a

HEK293 cell or introduced by recombinant means, and whereby the functional coupling of the taste receptor to the G_i protein activates a different signaling pathway [than promiscuous G proteins](See Ozeck et al., *Eur. J. Pharmacol.* 489:137-149 (2004)) than that mentioned by Li et al.

In the current invention, activation occur via a signaling cascade which does not involve calcium mobilization. In fact, as noted above, the subject taste receptors do not promote calcium mobilization in the absence of promiscuous G proteins (those expressed in the Li et al expression system). This is clear upon review of the Chandrashekar et al, *Cell* 100:703-711 (2000); and Nelson et al., *Cell* 106:381-390 (2001) references. Moreover, in the subject invention which measures G_i mediated activation of enzyme, the addition of PTX totally abolishes the effect of bitter, sweet and umami receptor on cAMP levels. (This is disclosed in the experimental section of the subject patent application as well as in Ozeck et al., *Eur. J. Pharmacol.* 489:139-149 (2004)). These results prove unequivocally that the subject assays monitor reactions which involve G_i mediated signaling cascades involving the inhibition of adenylyl cyclase activity and do not involve calcium mobilization.

Therefore, the Zoller patent application taken alone or in view of Li et al. is not suggestive of the claimed assays.

Claims 21, 30-32, 36 and 43 also stand rejected over the Zoller et al. application in view of Wu et al.

Zoller et al. has been discussed above. For the reasons set forth therein, this patent application does not render obvious the claimed methods.

The addition of Wu et al. also does not suggest the claimed methods. The Wu reference is cited based on its teachings relating to STC-1 cells which express GPCRs including T2R bitter taste receptors and which further express G-proteins including $G\alpha_{\text{gust}}$ and $G\alpha_{i2}$. The Examiner concludes that this reference suggests that T2Rs would functionally couple to the recited G_i proteins and allegedly further suggests the use of these receptors in conjunction with G_i proteins in assays that detect second messengers such as cAMP or MAPK.

Applicants respectfully disagree. To the contrary, review of the manuscript by Wu et al. shows expression of $G\alpha_{\text{gust}}$ and $G\alpha_{T2}$ (not $G\alpha_{i2}$). The manuscript by Wu et al. does not allow one of ordinary skill in the art to predict that the inventors could develop a functional and successful assay by monitoring G_i –signaling pathways such as those which monitor activation based on a decrease in cAMP production. By contrast, the manuscript by Wu et al. shows that STC-1 cells express T2Rs and that stimulation of these T2Rs lead to calcium mobilization. Again the Office Action improperly suggests the connection between calcium mobilization and cAMP production as a basis for obviousness (see page 13 3rd paragraph, lines 11-13, page 14 first paragraph lines 1 and 2 and page 15 first 2 lines). However, as explained above, in the subject invention the inventors instead show that taste receptor activation decreases the level of cAMP inside the cells and that this occurs through a totally different (calcium independent) signaling cascade and enzyme than the one used by promiscuous G proteins. The effects of cAMP reported in the present invention have no connection whatsoever with calcium mobilization since taste receptors do not promote calcium mobilization in the absence of promiscuous G proteins or their variants (see Chandrashekar et al., *Cell* 100:703-711 (2000) and Nelson et al., *Cell* 106:381-390 (2001)).

Also PTx treatment totally abolishes the effect of bitter, sweet and umami receptors or changes of cAMP levels (see our patent application and Ozeck et al., *Eur. Journal of Pharmacology* 489:139-149 (2004)). This result proves that the decrease in cAMP is due to activation of G_i proteins and inhibition of adenylyl cyclase activity and that it has nothing to do with calcium mobilization.

The Office Action also states that “*Wu et al teach that STC-1 cells endogenously express the G-proteins $G\alpha_{\text{gust}}$ and $G\alpha_{\text{T2}}$ as well as other T2R receptors. They further show that T2R and the said G-proteins functionally couple together so as to elicit the intracellular calcium response when a compound is used to activate the T2R receptor*” (page 14, 4th paragraph). The Office Action first states that from this information it is obvious that taste receptors should modulate cAMP levels (again because of the apparent connection between calcium mobilization and cAMP levels). In fact, nowhere in Wu et al. is there any data providing that the endogenously expressed T2Rs functionally couple to $G\alpha_{\text{gust}}$ and $G\alpha_{\text{T2}}$ (or any other G-proteins for that matter) to induce calcium mobilization. Moreover, co-expression of receptors and G-proteins within the same cell type is not an argument sufficient to prove functional coupling. Such an argument ignores the inherent unpredictability associated with the coupling of GPCRs to G proteins and the fact that GPCRs typically only functionally couple to some types of G proteins and not all G proteins (which encompasses a large group of structurally and functionally diverse proteins which are involved in different signaling pathways). For example, while it is known that taste receptor cells express $G\alpha_s$ (see Kusakabe et al., *Chem Senses* 25:525-531 (2000)); these taste receptors do not functionally couple to $G\alpha_s$ in vivo (see Figure 7 in Ozeck et al., *Eur. Journal of Pharmacology* 489:139-149 (2004)) or in vitro (see Figure 7 in Chandrashekar et al., *Cell*

100:703-711 (2000)). Therefore, the fact that a cell co-expresses a particular GPCR and G protein does not necessarily correlate to functional coupling.

Based on the foregoing, withdrawal of the obviousness rejection of claims 21, 30-32, 36 and 43 based on Zoller ('472 application) in view of Wu et al. is respectfully requested.

Claims 5-7, 14, 22, 44, 45 and 54 further stand rejected as assertedly being unpatentable over Zuker et al. in view of McDonaldson et al and Naur et al.

The Zucker application is discussed above. For the reasons set forth therein, this patent application does not suggest that the subject T1R or T2R receptors would functionally couple with G_i proteins and induce G_i mediated signaling pathways that can be monitored to identify T1R and T2R modulatory compounds.

With respect to the reference by Margolskee and Naor et al. the Office Action refers to Margolskee's review to suggest that "*it is well known in the art that certain G-proteins such as $G\alpha_q$ proteins functionally couple to T2R receptors...*" (Page 16, 1st paragraph). The review of Naor et al. points out that $G\alpha_q$ -coupled receptors promote MAP Kinase activation, in part through calcium mobilization and PKC activation. The Office Action combines these two apparent pieces of information in an effort to suggest that it is obvious that taste receptors activation would lead to MAPK activation: "*Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the method of Zucker et al. or Zoller et al. but use T2R receptors that functionally couple to $G\alpha_q$ proteins and to screen for modulatory compounds by using MAPK assay*" (page 16, 2^d paragraph). However, Applicants respectfully note that the statement in the Office Action with regard to $G\alpha_q$

coupling is simply not accurate. Indeed, all evidence in the literature point to the fact that taste receptors do not functionally couple to $G\alpha_q$ (see Figure 7 in Chandrashekar et al. *Cell* 100:703-711 (2000)). This is the main reason why investigators have had to resort to the use of promiscuous G proteins such as $G\alpha_{15}$, $G\alpha_{16}$ or their variants to functionalize T1Rs and T2Rs (see Chandrashekar et al. *Cell* 100:703-711 (2000); Nelson et al. *Cell* 106:381-390 (2001) and Li et al.). The apparent co-expression of taste receptors and $G\alpha_q$ in taste receptor cells (as described in the review by Margolskee) therefore is not proof of functional coupling. Since taste receptors do not couple to $G\alpha_q$, this reference could not predict that the inventor could have developed a functional assay using MAPK.

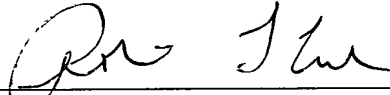
Therefore, based on the foregoing, withdrawal of the § 103 rejection of Claims 5-7, 14, 22, 44-45, and 54 based on Zucker and in view of MdDonaldson et al and Naur et al. is respectfully requested.

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket #100337.54281US).

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